



## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Nicolas VOUTE *et al.*Title: **SMALL DENSE MICROPOROUS SOLID SUPPORT  
MATERIALS, THEIR PREPARATION, AND USE...**

Appl. No.: 09/274,014

Filing Date: March 22, 1999

Examiner: D. Sorkin

Art Unit: 1723

**DECLARATION UNDER 37 CFR §1.132**Commissioner for Patents  
Washington, D.C. 20231**RECEIVED**

SEP 27 2002

**TC 1700**

Sir:

I, Egisto Boschetti, declare that:

1. I am a citizen of France. I am employed presently by CIPHERGEN Biosystems, Inc., the owner of U.S. application serial No. 09/274,014 ("the application"), which names me as a co-inventor. My research experience relates extensively to the field of chromatography, where I have contributed to the development of a number of original sorbents based on mineral oxides, organic polymers either synthetic or natural, and mixtures of them. Additionally, I have published extensively on the specific mechanisms of adsorption-desorption concepts for protein separation. A number of chromatography sorbents developed with my input currently are used in both small and large scale production of biomolecules for human therapy and research.

2. Regarding the application, I have reviewed both an Office Action, mailed April 3, 2002, and various documents cited there. The Office Action states that U.S. patent No. 5,015,373 to Carr *et al.* ("Carr"), in itself or in view of Girot *et al.*, U.S. patent No. 5,445,732 ("Girot"), would have rendered obvious the claims of the application.

3. Based on Carr, the examiner takes the position that one of ordinary skill in the chromatography art would have considered it obvious to make the porosity of beads less than 30%, as the claims, I understand, now require. The examiner apparently grounds his position on Carr's statement that, for such beads, "surface area and pore volume decrease with increasing firing temperature" (column 13, lines 37-39) and also that sintering temperature

for beads can range for 100-1500° C (column 8, lines 20-29). The examiner notes that Carr discloses a specific example of beads, with a porosity of 31%, that were obtained at a firing temperature of 900°C. Given Carr's disclosure of a broad temperature range of 100-1500°C, and his teaching that porosity decreases with increasing firing temperature, the examiner concludes that Carr would have suggested beads with a porosity of less than 30%. In this regard he argues that "it would have been obvious to one of ordinary skill in the art to have discovered an optimal or workable range of porosity" (paragraph bridging pages 2 and 3).

4. It is my opinion, speaking as an expert in the field of chromatography, that Carr does not lead a skilled artisan to produce mineral oxide beads with a pore volume of less than 30%. To the contrary, Carr suggests to me that pore volume and pore size both should be maximized. Larger pore volumes and pore sizes are in keeping with Carr's use of the beads to separate proteins. The state of the art prior to the present invention taught that protein separation methodologies should permit entry into the pores of the medium by the proteins to be separated, and this is in total agreement with repeated suggestions in Carr that these parameters be maximized, as detailed below. Increased pore diameter and pore volume together allow more of the proteins that are to be separated to diffuse freely into the internal void volume.

5. Carr not only fails to suggest mineral oxide beads with a pore volume less than 30%, but also does not disclose any advantage associated with such beads; hence a reader of Carr would not be motivated to produce such beads. By contrast, the beads described in the application have several advantages, disclosed on pages 9, 10, 16 and 17 of the application. First, a pore volume of less than 30% allows polymers to be rooted in the pores, so that they are firmly anchored during the interaction of the desired molecules that occurs on the external surface area of the beads. Second, small pore volumes prevent molecules from diffusing inside the beads, so that they leave the column with the source liquid. When molecules diffuse inside the pores, they can be eliminated only by using long and repeated washings, a distinct disadvantage. Finally, the smaller the pore volume, the larger the particle density. Density is an advantage particularly in the context of fluidized bed technologies, one of the intended uses of the present beads. None of these unexpected advantages of the present beads are alluded to in Carr. These advantages are discussed in greater detail in the paragraphs which follow.

6. The examiner's rationale presumes that the prior art illustrated by Carr would have prompted the skilled artisan to pursue the goal of reducing pore volume for a chromatography medium of the sort prescribed in the claims of the application. Yet the statements that the examiner highlights from Carr neither suggest, from context, any advantage to this goal nor provide guidance to this end. Addressing the latter point first, I would emphasize that: (A) the cited art alludes to multiple parameters that affect pore volume and pore diameter and (B) there is no guidance on permutations of these parameters, including but not limited to sintering temperature, that would yield a pore volume of less than 31%, which is the value in the example invoked by the examiner.

7. More particularly, Carr identifies at least two parameters that affect pore volume, pore diameter, and surface area: firing temperature ("the surface area and pore volume decrease with increasing firing temperature") and colloid size ("larger colloids produce fired spherules with larger pore diameters and pore volumes" – col. 14, lines 4-5). Thus, pore volume can be decreased by increasing the firing temperature and/or by decreasing the colloid size. Conversely, the two parameters can be selected so that they counteract one another with respect to pore volume, *i.e.*, a higher firing temperature and a larger colloid size both increase pore diameter, but the two parameters have opposite effects on pore volume and surface area, as shown in Tables I and II of Carr.

8. Carr teaches that pore volume should be maximized, and not controlled to values of less than 30%. Thus, Carr states that "the data summarized in Table 1 show that it is possible to increase the average pore diameter by increasing the firing temperature from 400° to 900°C. The surface area and pore volume decrease with increasing firing temperature. *Chromatographic activity of the ZrO<sub>2</sub> spherules is determined by the parameters of the surface area, average pore diameter and pore volume. Accordingly, **the appropriate firing temperature is selected***" (column 13, lines 35-42, emphasis added). These explicit teachings by Carr suggest larger pore volumes, not smaller, and the intended use for Carr's media likewise would have pointed the skilled artisan toward larger pore volumes. Thus, Carr discloses a media for separation of proteins, particularly antibodies, and Carr's methodology entails entry into the pores of the medium by the proteins to be separated. Increased pore diameter and pore volume together allow more of the proteins that are to be separated to diffuse freely into the internal void volume, thereby reaching all available surface area, which

also should be maximized. Accordingly, Carr's intended use militates in favor of maximizing both pore volume and pore diameter.

9. The examples of Carr clearly select firing temperature and colloid size so as to maximize porosity and pore size, typically by using a firing temperature of 600°C. A further emphasis on increasing both pore volume and pore size is found in Example 8, which discloses that "centrifugation, removal of the supernatant, and redispersion of the colloidal ZrO<sub>2</sub> starting material results in increases in the average pore diameter, pore volume and surface area of the fired spherules...regardless of mechanism, the centrifugation treatments describes in Examples 6-8 provide a method of preparing spherules with *increased* average pore diameter, *pore volume* and surface area relative to spherules prepared from untreated colloidal ZrO<sub>2</sub> sols" (column 16, lines 13-17, emphasis added).

10. All of Carr's examples that relate to coated particles employ particles prepared according to Example 2. Example 2 fires the ZrO<sub>2</sub> spherules at 600°C. As shown in Table 1, these particles have a pore volume of 36%. There is no suggestion to fire at a higher temperature in order to reduce this pore volume. Indeed, Carr *et al.* counsel that both pore volume and pore size should be maximized, as noted above. Certainly, there is no suggestion to modify the firing temperature in order to produce a matrix with a pore volume of less than 30%.

11. In addition to providing no guidance on the combination of parameters to be used in achieving a goal of pore volume less than 30%, Carr also does not suggest, from context, any advantage associated with the achievement of this goal. By contrast, the invention claimed in the application produces particles with low pore volume by firing at high temperatures, in the range of 900°C to 1500°C, and preferably between 1000°C and 1400°C, so as to melt the submicroparticles together and reduce the particle diameter and reduce the pore volume to less than about 30%. This low pore volume material is essential to firmly anchor the interactive polymer network on the beads - to provide a polymer network that is firmly rooted in the pores. The emphasis in Carr is on large pore diameters and pore volume, to allow free access to the interior of proteins to be separated. The pore volume of less than 30%, according to the invention claimed in the application, is one that is just large enough to allow polymers to be rooted in the pores. Thus, the prescribed pore volume is related directly to the disclosed purpose of firmly anchoring an interactive polymer network

that is crosslinked in the pores. The interaction of the desired molecules occurs on the external surface area of the beads due to the rooted polymers. The surface layer of polymer interacts with desired macromolecules and is held stably in place because it is part of a network that is crosslinked in the pores of the media. This is described in the paragraph bridging pages 9 and 10 of the application:

the pore volume is left just large enough to allow polymers to be rooted in the pores, and these rooted polymers layer on the external surface of the beads where the interaction with the macromolecules occurs. The resulting layer of polymers, or interactive polymer network, is stable and remains in place. The interaction of the desired molecules occurs on the external surface area of the beads due to the rooted polymers.

The role of the pores is to provide just enough space inside the beads to anchor the polymer. The crosslinked polymer will be exposed only at the external bead surface where the interaction with large macromolecules occurs. "Rooting" of the polymer is needed because the external layer of polymer responsible for the interaction with macromolecules must be stable. According to the invention claimed in the application, it is stabilized in place as a tree that is rooted in its substrate. Carr does not even hint at this advantage.

12. A large pore volume not only is much less effective in stably rooting the polymer but also allows molecules to diffuse inside the beads, instead of leaving the column with the source liquid. When molecules diffuse inside the pores, they can be eliminated only by using long and repeated washings. The diffusion of molecules within the secondary network created by the polymer is not completely prevented, but is significantly reduced in particular with mineral oxides possessing pore volumes less than 30%, and it is reduced even more when the pore volumes are less than 25% or 15%, two preferred embodiments of my invention. In particular, when pore volume is less than 15%, diffusion of molecules is so restricted that it is almost non-existent. A small pore volume thus results in a second advantage that Carr does not suggest.

13. Finally, there is yet another advantage provided by the small pore volume, in accordance with the invention claimed in the application: the smaller the pore volume, the larger the particle density. Density is an advantage particularly in the context of fluidized bed technologies, one of the intended uses of the present beads. This third advantage also is not suggested by Carr.

14. From what I have said in the preceding paragraphs, it is apparent that Carr discloses neither how to achieve small pore volumes nor any reason for doing so. Carr also fails to teach an interactive, crosslinked polymer network that fills the pores and is coated on the external surface of the mineral oxide matrix, such that subsequent interaction with macromolecules occurs on the external surface area of the support, as is the case with the invention claimed in the application. The polymer in Carr coats but does not fill the pores. This is evident from the statement, at the bottom of column 5 of the reference, that the  $ZrO_2$  spherules of Carr also can be employed "to immobilize bioactive materials for a variety of purposes, including catalysis, analysis, affinity chromatography and synthetic transformations. Bioactive materials can be strongly sorbed onto the exterior and *interior surfaces of* both the uncoated and *the polymer-coated  $ZrO_2$  spherules*, while retaining a large percentage of their initial bioactivity. Useful biomaterials include proteins such as enzymes and antibodies" (emphasis added). The fact that there are "interior surfaces of the polymer-coated  $ZrO_2$  spherules" clearly shows that the beads are different than the beads according to my invention in which an interactive crosslinked polymer network fills the pores and is coated on the external surface of the mineral oxide matrix, so that subsequent interaction with macromolecules occurs on the external surface area of the support.

15. The examiner adds Girot to Carr in rejecting some of the claims. I am a co-inventor of Girot and, hence, am in a particularly good position to shed light on its disclosure. The purpose of the subject matter described in this patent is to have a hydrogel fill the pores of mineral oxides entirely, while allowing even macromolecules still to diffuse inside. Therefore, the pore volume is made as large as possible, to maximize the binding capacity of the final material. This approach contrasts sharply with that described in the application. The latter strives to foreclose access to the pore volume by the molecules in the source solution, and it uses a small pore volume in order that a crosslinked polymer can be stably rooted in the matrix and that interactions with macromolecules should occur only on the external surface of the bead.

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I hereby declare that all the statements made herein of my known knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so made punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United

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States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

September 13, 2002

Date



Egisto Boschetti